

Autonomic compensation to simulated hemorrhage monitored with heart period variability

William H. Cooke, PhD; Caroline A. Rickards, PhD; Kathy L. Ryan, PhD; Victor A. Convertino, PhD

Objective: To test the hypothesis that components of heart period variability track autonomic function during simulated hemorrhage in humans.

Design: Prospective experimental laboratory intervention.

Setting: Human physiology laboratory.

Subjects: A total of 33 healthy, nonsmoking, volunteer subjects (23 men, ten women).

Interventions: Progressive lower body negative pressure was applied in 5-min stages until the onset of impending cardiovascular collapse.

Measurements and Main Results: The electrocardiogram, beat-by-beat finger arterial pressure, and muscle sympathetic nerve activity from the peroneal nerve were recorded continuously. Pulse pressure was calculated from the arterial pressure waveform and used as an estimate of relative changes of central blood volume. Heart period variability was assessed in both time and frequency domains. Application of lower body negative pressure caused progressive reductions of R-R interval and pulse pressure

and progressive increases of muscle sympathetic nerve activity. Arterial pressures changed minimally and late. R-R interval time domain variability measures and spectral power at the high frequency (0.15–0.4 Hz) decreased progressively with lower body negative pressure ($p < .001$). Both R-R interval high-frequency power and time domain variability measures correlated inversely with muscle sympathetic nerve activity and directly with pulse pressure (all amalgamated $R^2 > .88$, all $p \leq .001$).

Conclusions: Components of heart period variability track early compensatory autonomic and hemodynamic responses to progressive reduction in central blood volume. Such analyses, interpreted in conjunction with standard vital signs, may contribute to earlier assessments of the magnitude of blood volume loss during hemorrhage. (Crit Care Med 2008; 36:1892–1899)

KEY WORDS: central hypovolemia; lower body negative pressure; stroke volume; pulse pressure; blood pressure; remote medical monitoring

Hemorrhage is one of the leading causes of death for both civilian trauma patients and battlefield casualties (1). Despite this, first responders have no tools to assess volume status before definitive medical intervention. Standard vital signs such as arterial pressure, heart rate, and arterial oxygen-hemoglobin saturation remain stable (if somewhat high in the case of heart rate)

during hemorrhage, until the onset of cardiovascular decompensation, and therefore provide little information on early changes of volume status and subsequent identification of the need for prompt intervention in severely injured patients (2).

Importantly, compensation to severe hemorrhage is driven by the autonomic nervous system. Loss of blood volume triggers withdrawal of parasympathetic and activation of sympathetic neural activity to defend against decreases of arterial pressure (2). Consequently, arterial pressure may fail to provide an early indication of hemorrhage severity because it is maintained fairly constant during the early stages of mild to moderate hemorrhage through autonomic neural mechanisms that increase peripheral vascular resistance and heart rate. By the time arterial pressures fall to dangerous levels (which can occur quickly), it may already be too late to apply effective lifesaving interventions. Instead of monitoring regulated variables (i.e., outcomes), such as arterial pressure, medical personnel need the capability to monitor responses of

controlling mechanisms—in this case, autonomic neural function.

Frequency domain analysis of heart period variability has been used as a technique to assess autonomic function in patients monitored in an intensive care unit (ICU), and differences in frequency characteristics were found to be associated with patient outcome (3, 4). Norris et al. (5) have demonstrated the ability of time domain measures of heart rate variability to accurately predict death from traumatic injury up to 12 hrs before it occurs. Grogan et al. (6) have suggested, based on thousands of individual ICU patient records, that *volatility*, a derivation of beat-by-beat heart rate variability in which heart rate is sampled every 1 to 4 secs, shows promise as a new vital sign for ICU patient monitoring. We have shown previously, through frequency-domain analysis of heart period (R-R interval) variability in prehospital trauma patients, that the high-frequency-to-low-frequency ratio derived from a Fourier transform (HF/LF) is higher in patients who eventually die compared with patients who survive traumatic injury (7,

From the University of Texas at San Antonio, San Antonio, TX (WHC); and the U.S. Army Institute of Surgical Research, Fort Sam Houston, TX (CAR, KLR, VAC).

The authors have not disclosed any potential conflicts of interest.

Supported, in part, by the U.S. Department of Defense, Medical Research and Materiel Command.

The views expressed herein are the private views of the authors and are not to be construed as representing those of the U.S. Department of Defense or the U.S. Department of the Army.

Address for requests for reprints to: William H. Cooke, PhD, University of Texas at San Antonio, Department of Health and Kinesiology, One UTSA Circle, San Antonio, TX 78249. E-mail: william.cooke@utsa.edu

Copyright © 2008 by the Society of Critical Care Medicine and Lippincott Williams & Wilkins

DOI: 10.1097/CCM.0b013e3181760d0c

Report Documentation Page				Form Approved OMB No. 0704-0188	
Public reporting burden for the collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden, to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to a penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number.					
1. REPORT DATE 01 JUN 2008		2. REPORT TYPE N/A		3. DATES COVERED -	
4. TITLE AND SUBTITLE Autonomic compensation to simulated hemorrhage monitored with heart period variability				5a. CONTRACT NUMBER	
				5b. GRANT NUMBER	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S) Cooke W. H., Rickards C. A., Ryan K. L., Convertino V. A.,				5d. PROJECT NUMBER	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) United States Army Institute of Surgical Research, JBSA Fort Sam Houston, TX 78234				8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)				10. SPONSOR/MONITOR'S ACRONYM(S)	
				11. SPONSOR/MONITOR'S REPORT NUMBER(S)	
12. DISTRIBUTION/AVAILABILITY STATEMENT Approved for public release, distribution unlimited					
13. SUPPLEMENTARY NOTES					
14. ABSTRACT					
15. SUBJECT TERMS					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT UU	18. NUMBER OF PAGES 8	19a. NAME OF RESPONSIBLE PERSON
a. REPORT unclassified	b. ABSTRACT unclassified	c. THIS PAGE unclassified			

8). These differences are detectable at a point in time when standard vital signs such as mean arterial pressure, heart rate, and arterial oxygen-hemoglobin saturation are not different between patient groups.

If some component of heart period variability does in fact track autonomic compensation to reduced central blood volume (hemorrhage), it would be an attractive candidate for use in noninvasive monitoring systems for trauma patients and combat casualties. However, scientists and clinicians are split as to whether heart period variability does (9, 10) or does not (11, 12) reflect modulation by the autonomic nervous system. Pagani et al. (13) introduced the concept that the ratio of LF/HF R-R interval spectral power calculated from frequency domain analysis provides an indication of sympathetic vs. parasympathetic cardiac control. This contention derives from observations that R-R interval low-frequency oscillations (RRI_{LF} ; 0.04–0.15 Hz) are modulated, importantly (but not exclusively), by sympathetic neural traffic (10, 13). However, both Kingwell et al. (14) and Saul et al. (15) failed to demonstrate significant correlations between directly measured muscle sympathetic nerve activity (MSNA) and RRI_{LF} in research volunteers at rest. Furthermore, induced sympathetic activation with nitroprusside infusion sufficient to decrease arterial pressures by about 15 mm Hg revealed nonsignificant correlations between MSNA and RRI_{LF} (15). Although this observation suggests a lack of association between these variables, pharmacologic reduction of arterial pressure does not represent the same physiologic condition as the progressive reduction of central blood volume that accompanies severe hemorrhage. However, severe hemorrhage in humans cannot be studied in a controlled laboratory environment.

We have proposed the use of lower body negative pressure (LBNP) as a research tool to simulate the progression from mild to severe hemorrhage in humans during the preshock state (16). It has been known for some time that progressive LBNP reduces heart rate variability (17, 18) and increases MSNA (19). However, a robust assessment of the direct relationship between heart rate variability and MSNA during progressively severe reductions in central blood volume (LBNP levels of >45 mm Hg) has been limited because recordings of sympathetic traffic (recorded most commonly

from tibial or peroneal nerves at the knee) are often lost due to interference of the microelectrode by increases of negative chamber pressure. Negative pressures on the order of –60 mm Hg and above induce physiologic responses similar to blood loss of >1000 mL (16). Therefore, the issue of whether changes of heart period variability reflect increases of MSNA during severe central hypovolemia remains to be resolved before heart period variability can be considered as a diagnostic tool for assessing severity of hemorrhage.

To address this issue, we measured MSNA from peroneal nerves directly, using the microneurography technique, in a cohort of healthy human volunteers during simulated hemorrhage with LBNP. The purpose of this study was to test the hypothesis that components of heart period variability track autonomic function during progressive central hypovolemia of a magnitude estimated to be similar to that of severe hemorrhage.

MATERIALS AND METHODS

Subjects. A total of 33 nonsmoking subjects (23 men, ten women) volunteered to participate in this study (age, 31 ± 2 yrs; height, 174 ± 2 cm; weight, 80 ± 3 kg). Before inclusion, all subjects underwent a medical history and physical examination by a physician to ensure they had no previous or current medical conditions that might preclude their participation. To ensure female subjects were not pregnant, all potential female subjects underwent a urine test within 24 hrs of experimentation. All subjects maintained their normal sleep patterns, refrained from exercise, and abstained from caffeine and other autonomic stimulants, including prescription or nonprescription drugs, for ≥ 24 hrs before the study. Subjects received a verbal briefing and written descriptions of all procedures and risks associated with the study and were made familiar with the laboratory, the protocol, and procedures. Subjects were encouraged to ask questions of the investigators, and then they signed an informed consent form that had been approved by the Institutional Review Board for the protection of human subjects in research from Brooke Army Medical Center and the U.S. Army Institute of Surgical Research, Fort Sam Houston, TX.

Experimental Protocol. Subjects were instrumented with a standard four-lead electrocardiograph (ECG) to record cardiac electrical potentials, and a finger cuff to record beat-by-beat finger arterial pressure (Finometer Blood Pressure Monitor, TNO-TPD Biomedical Instrumentation, Amsterdam, The Netherlands). MSNA was recorded directly from peroneal nerves with the microneurography technique

(20). Central hypovolemia was induced by application of LBNP to simulate, as closely as possible in healthy human volunteers, the hemodynamic challenges associated with severe hemorrhage (16). Subjects were positioned supine within an airtight chamber that was sealed at the level of the iliac crest by way of a neoprene skirt. Because injured patients do not breathe at a fixed rate, we did not attempt to control breathing frequency, and subjects were allowed to breathe spontaneously. Each subject underwent exposure to progressive LBNP until the point of impending cardiovascular collapse. The LBNP protocol consisted of a 5-min control period (baseline) followed by 5 mins of chamber decompression at –15, –30, –45, and –60 mm Hg, and then additional increments of –10 mm Hg every 5 mins until the onset of cardiovascular collapse followed by a 10-min recovery period. Cardiovascular collapse was defined by one or a combination of the following criteria: a) onset of presyncopal symptoms such as gray out, b) a precipitous fall in systolic pressure of >15 mm Hg or a sudden bradycardia, c) progressive diminution of systolic pressure to <70 mm Hg, and d) voluntary subject termination due to discomfort from symptoms such as sweating, nausea, or dizziness.

Data Analysis. Data were sampled at 500 Hz and digitized to computer (WINDAQ, Dataq Instruments, Akron, OH). The last 3 mins of data from each LBNP stage and the 3 mins just before cardiovascular collapse were extracted for analysis. Individual R-waves generated from the ECG were marked at their occurrence in time and used for subsequent identification of systolic and diastolic pressures generated from the Finometer. Pulse pressures were calculated from the difference between systolic and diastolic pressure and used as an estimate of changes in stroke volume (21). All analyses were performed with commercially available data analysis software (WinCPRS, Absolute Aliens, Turku, Finland).

For representation of heart period variability in the time domain, we calculated R-R interval SD (RRISD) and the percentage of adjacent R-R intervals that varied by ≥ 50 msec (pNN50). For representation of heart period variability in the frequency domain, R-R intervals were replotted using linear interpolation and resampled at 5 Hz. Data then were passed through a low-pass impulse response filter with a cutoff frequency of 0.5 Hz. Data sets were submitted to a Fourier transform with a Hanning window. The magnitude of R-R interval oscillations were quantified by calculating the power spectral density for the signal (total; 0.04–0.4 Hz). Signal areas were separated into high-frequency (RRI_{HF} ; 0.15–0.4 Hz) and low-frequency (RRI_{LF} ; 0.04–0.15 Hz) bands. To account for potentially large intersubject variability common with frequency analyses, we also normalized RRI_{HF} and RRI_{LF} to total power by dividing both RRI_{HF} and RRI_{LF} by the total power (the sum of RRI_{HF} and RRI_{LF}) (22).

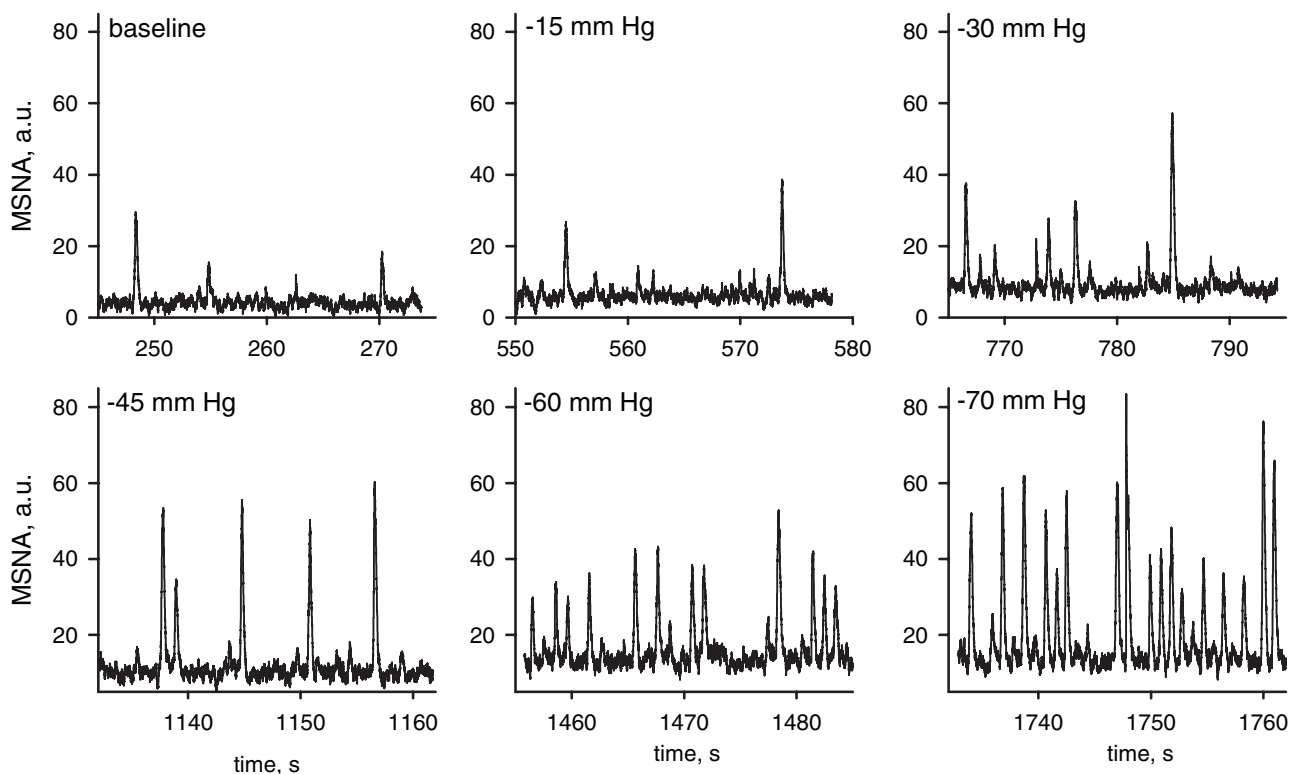


Figure 1. Muscle sympathetic nerve activity (MSNA) from a peroneal nerve is shown for one subject during progressive increases of lower body negative pressure.

Statistical Analysis. A one-way analysis of variance for repeated measures was used for comparison of dependent variables across treatments (levels of LBNP). A two-way analysis of variance for repeated measures was used for comparison of dependent variables across gender groups (men vs. women) and treatments (levels of LBNP). Duncan's mean separation procedure was employed for levels of LBNP when the probability of LBNP main effects was $<.05$. Amalgamated Pearson correlation coefficients were calculated among variables of interest. The probabilities of observing chance effects due to our experimental manipulations are presented as exact p values (23). All data are expressed as mean \pm SE unless specified otherwise.

RESULTS

Average time to cardiovascular collapse was 1816 ± 60 secs, revealing that a majority of subjects experienced symptoms late into the -60 mm Hg stage to early into the -70 mm Hg ($n = 33$). No subject reported feeling anxiety or discomfort associated with LBNP.

Application of LBNP often causes the microneurography electrode tip to shift and move beyond the recording region within the peroneal nerve fascicle. Such minute changes in electrode position degrade the signal in some subjects to the

point where spontaneous bursts of nerve traffic are no longer observable. We were able to maintain good MSNA recordings at lower levels of negative pressure, but signal quality tended to degrade as chamber pressure increased. For this reason we did not quantify total MSNA (burst area multiplied by the number of bursts) but, rather, quantified MSNA as burst rate (bursts per 100 heart beats). Although our protocol took each subject to cardiovascular collapse, we only included data from subjects with good MSNA recordings for correlations with heart period variability metrics. MSNA was maintained throughout cardiovascular collapse in 12 subjects (seven men and five women), and therefore, the unequal sample sizes we report for each LBNP level represent primarily loss of MSNA before cardiovascular collapse. Of the 12 subjects in whom nerve recordings were maintained until cardiovascular collapse, one experienced symptoms during -60 mm Hg, six during -70 mm Hg, and five during -80 mm Hg. An example of an MSNA recording maintained at high negative pressures is shown for one subject in Figure 1. MSNA increased proportionately with increasing levels of LBNP in both men ($F = 8.391$, $p < .001$) and women ($F = 3.388$, $p =$

.011). The primary physiologic responses and the number of subjects studied at each LBNP stage are shown in Table 1.

RRI_{LF} and RRI_{HF} correlated poorly with MSNA at baseline before application of LBNP ($r = .12$ for RRI_{LF} and MSNA; $r = .10$ for RRI_{HF} and MSNA; $n = 33$). Application of LBNP caused progressive decreases of R-R interval and pulse pressure but changed systolic and diastolic pressures minimally. Systolic pressure began to decrease at -45 mm Hg, with little change in either diastolic or mean pressure. Respiratory rates in all 33 subjects at baseline (15 ± 1 breaths/min) did not differ statistically ($F = .532$, $p = .600$) from those at the maximal level of LBNP (15 ± 1 breaths/min) and were maintained at all times within the high-frequency range (.15 to .4 Hz).

Application of LBNP caused progressive reductions of $RRISD$, $pNN50$, and RRI_{HF} but did not affect RRI_{LF} ($p = .15$). $pNN50$ and RRI_{HF} were reduced at -30 mm Hg (both $p < .05$), whereas $RRISD$ was reduced at -45 mm Hg ($p = .04$) as compared with baseline. These patterns were not influenced by gender. Progressive reductions in RRI_{HF} were observed in both men ($F = 4.76$, $p < .001$) and women ($F = 3.99$, $p < .001$), but RRI_{LF} was unaltered across LBNP in both

Table 1. Hemodynamic and sympathetic neural responses to LBNP

Variable	Baseline n = 33	LBNP 15 n = 33	LBNP 30 n = 24	LBNP 45 n = 19	LBNP 60 n = 14	LBNP 70 n = 9	LBNP 80 n = 5	Recovery n = 10
RRI, ms	975 ± 23a	951 ± 22b	889 ± 28c	804 ± 38d	702 ± 47e	633 ± 53f	605 ± 55f	1020 ± 58a, b
HR, bpm	63 ± 1.4a, b	64 ± 1.4a, b	70 ± 2.6a, c	77 ± 3.3c	89 ± 5.4d	99 ± 7.8e	103 ± 8.5e	61 ± 3.5b
SAP, mm Hg	131 ± 1.7a, b	132 ± 1.9a, b	127 ± 2.1b, c	122 ± 2.7c, d	119 ± 4.2d	112 ± 8.1e	116 ± 5.1d, e	136 ± 5.1a
DAP, mm Hg	77 ± 1.0a	78 ± 1.1a, b	78 ± 1.1a, b	78 ± 1.2a, b	79 ± 1.8a, b, c	77 ± 3.8a	83 ± 3.0c, d	81 ± 2.8a, b, c
MAP, mm Hg	98 ± 1.2a	98 ± 1.1a	96 ± 1.1a	95 ± 1.4a, b	94 ± 2.5b, c	89 ± 5.1d	94 ± 3.2e	102 ± 3.5a
PP, mm Hg	54 ± 1.4a	54 ± 1.5a	49 ± 1.9b	44 ± 2.4c	40 ± 3.2d	34 ± 5.1e	33 ± 4.6e	53 ± 2.3a
MSNA, b/100	21 ± 2.0a	27 ± 2.3a, b	32 ± 2.6b, c	37 ± 3.5c, d	42 ± 3.1d	41 ± 3.8d	44 ± 5.7d	26 ± 5.0a, b

Values are means ± SE; RRI, R-R interval; HR, heart rate; SAP, systolic arterial pressure; DAP, diastolic arterial pressure; MAP, mean arterial pressure; PP, pulse pressure; MSNA, muscle sympathetic nerve activity expressed as the number of bursts per 100 heart beats; LBNP, lower body negative pressure. Differences between means within a row that share the same letter are not distinguishable statistically ($p \geq .05$).

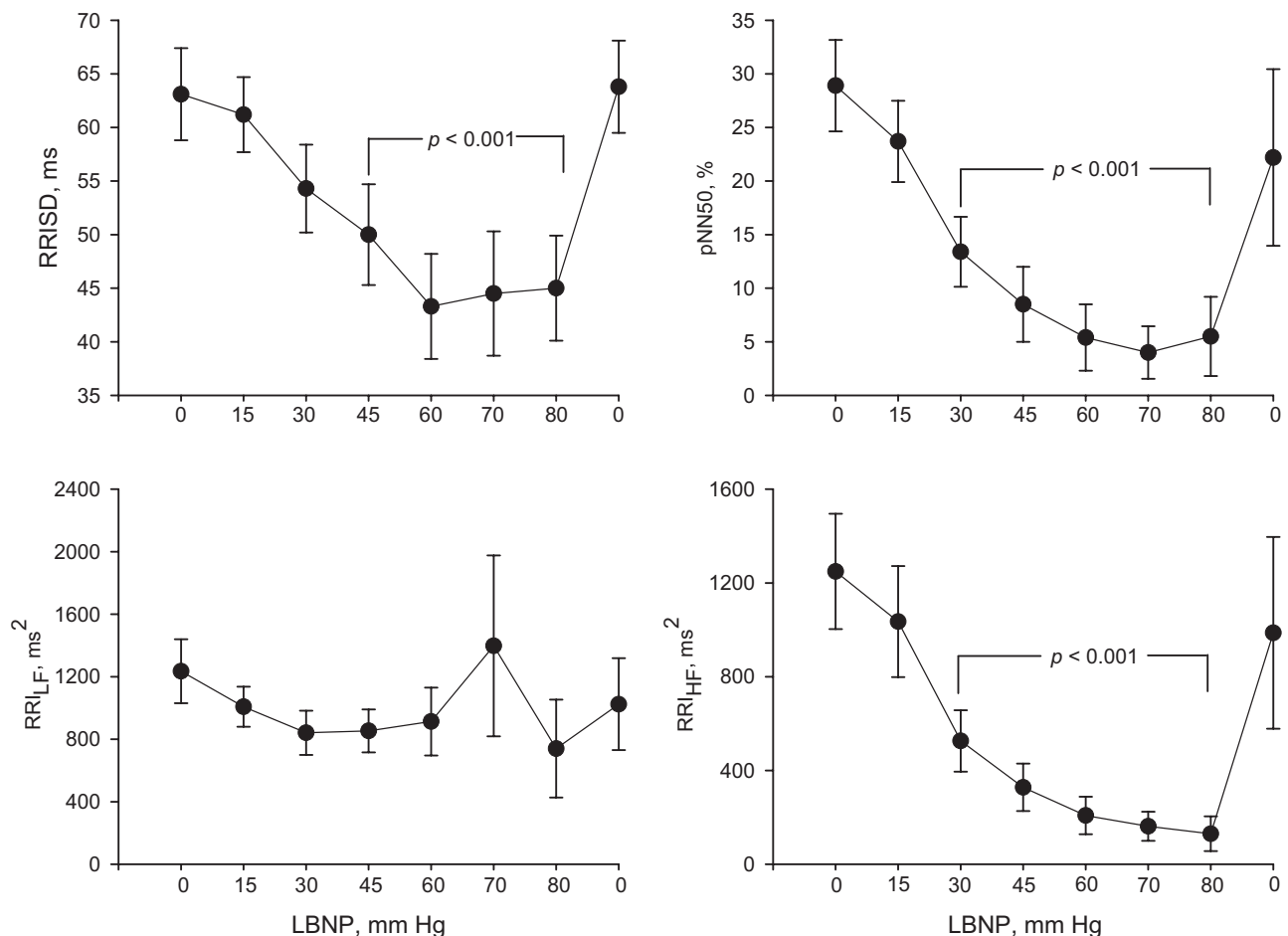


Figure 2. Group averages are shown ± SE for R-R interval SD ($RRISD$), the percentage of adjacent R-R intervals that varied by ≥ 50 msec ($pNN50$), and R-R interval oscillations derived from a Fourier transform at the low (RRI_{LF}) and high (RRI_{HF}) frequencies. Data identified with brackets show differences from baseline. LBNP, lower body negative pressure.

groups ($F \leq 1.232$, $p \geq .324$). There were no statistically distinguishable differences in responses of heart period variability and MSNA between men and women ($F \leq 1.134$, $p \geq .442$). Changes of time and frequency domain measures of heart period variability with LBNP for the entire group of 33 subjects are shown in Figure 2.

After normalization of R-R interval oscillations to total power, RRI_{LF} increased and RRI_{HF} decreased progressively with LBNP and were significantly different from baseline at -45 mm Hg ($p < .001$).

Figure 3 shows associations among time and frequency domain measures expressed in absolute units vs. MSNA. In both time and frequency domains, analyses of

components of heart period variability demonstrated high, inverse correlations with MSNA ($R^2 \geq .95$), with the exception of a low correlation between MSNA and RRI_{LF} calculated in absolute units ($R^2 = .09$).

High amalgamated correlation coefficients, as shown in Figure 3, may sometimes mask a high rate of fairly low individual correlations. To address this issue,

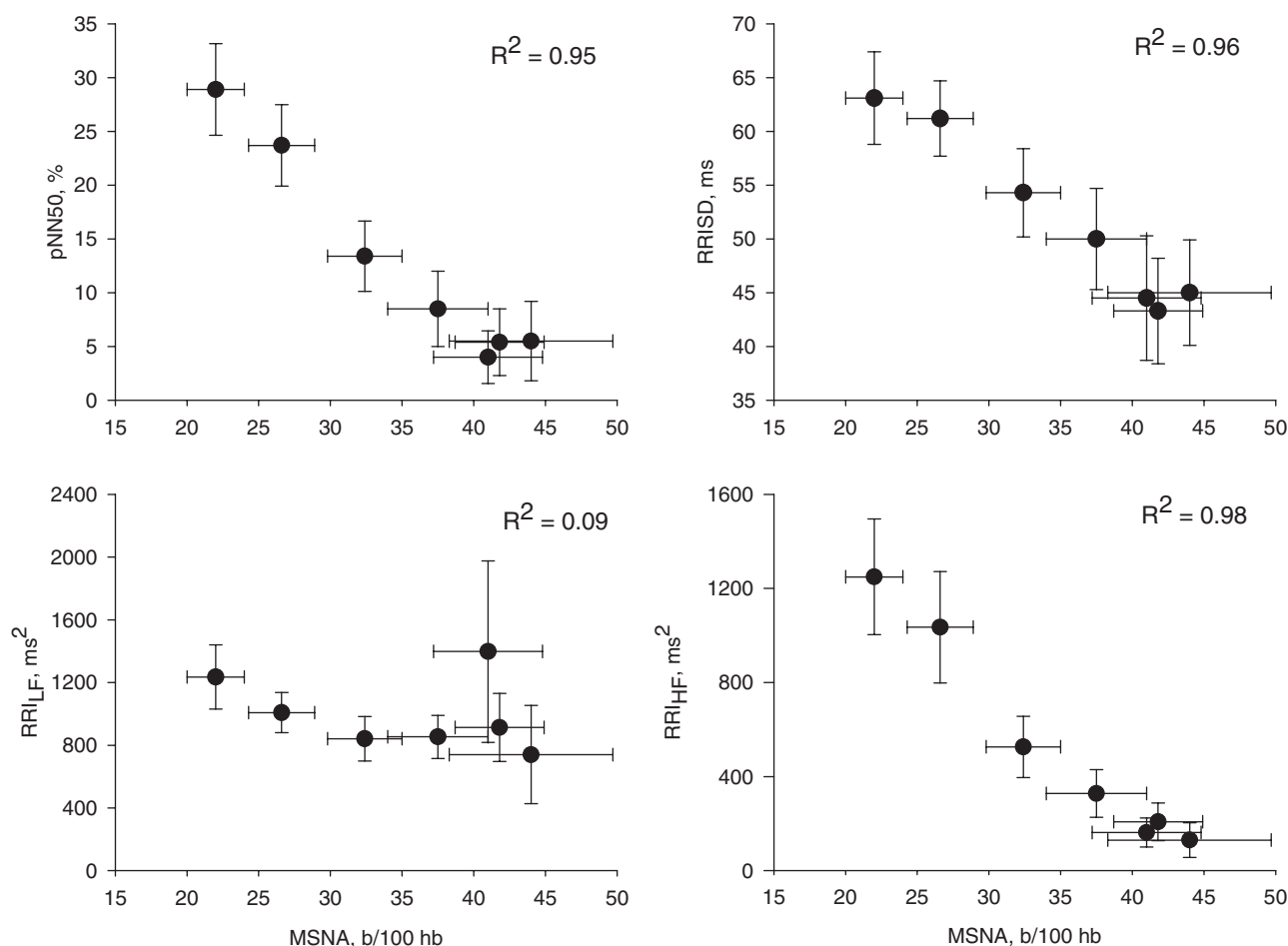


Figure 3. Group averages for time and frequency domain variables \pm SE are plotted against muscle sympathetic nerve activity (MSNA), with amalgamated correlation coefficients shown in the upper right corner of each panel. *RRISD*, R-R interval SD; *RRI_{LF}*, R-R interval oscillations derived from a Fourier transform at the low frequency; *RRI_{HF}*, R-R interval oscillations derived from a Fourier transform at the high frequency; *pNN50*, the percentage of adjacent R-R intervals that varied by ≥ 50 msec.

Table 2. Average individual correlations among various heart rate variability metrics and MSNA during LBNP from a sample of subjects from the study cohort who provided data up to -70 mm Hg ($n = 9$)

Variables	Mean Pearson r	% individual $r \geq 0.70$
RRI HF	.55 \pm .09	44%
RRI LF	.32 \pm .07	0%
RRI HFnu	.65 \pm .08	55%
RRI LFnu	.66 \pm .07	55%
RRISD	.64 \pm .07	44%
pNN50	.65 \pm .06	55%

Values are means \pm SE. MSNA, muscle sympathetic nerve activity; RRI HF, R-R interval high frequency power; RRI LF, R-R interval low frequency power; RRI HFnu, normalized high frequency power; RRI LFnu, normalized low frequency power; RRISD, R-R interval standard deviation; pNN50, the percentage of adjacent R-waves that vary by 50 msec or more.

we chose a sample cohort of subjects who provided MSNA data up to -70 mm Hg ($n = 9$) and ran individual correlations among primary associations of interest. We chose this sample cohort because including data through -70 mm Hg provided a minimum number of data sets for robust calculation of individual correlation coefficients. We considered a correlation of $\geq .7$ to be strong. Average individual correlations with the percentage of individuals with correlations of $\geq .7$ are shown in Table 2.

RRISD, pNN50, RRI_{HF}, and normalized RRI_{HF}, correlated directly with decreases of pulse pressure during LBNP (all $R^2 \geq .88$). Normalized RRI_{LF} correlated inversely with pulse pressure ($R^2 = .94$), but absolute RRI_{LF} did not ($R^2 = .005$).

DISCUSSION

We submitted a group of healthy human volunteers to progressive reduction in central blood volume to determine

whether heart period variability provides insight into autonomic responses to progressive central hypovolemia. The primary new findings from this study are: 1) time- (RRISD, pNN50) and frequency- (RRI_{HF}) derived components of heart period variability decrease progressively with increases of MSNA, and 2) RRI_{LF} does not change. Our results have implications for monitoring of bleeding patients using a simple ECG and provide insight into the utility of time and frequency domain analyses of cardiac inter-beat intervals as markers of autonomic and hemodynamic compensation to central blood volume reductions and early indicators of hemorrhage severity.

The report of the Post Resuscitative and Initial Utility of Life Saving Efforts (PULSE) trauma work group, sponsored, in part, by the National Heart, Lung, and Blood Institute, revealed that hemorrhage accounts for a large proportion of deaths due to traumatic injury (1). Be-

cause a full 40% of traumatically injured patients die before they reach a hospital, one recommendation of the work group was to improve or develop new approaches to monitoring bleeding patients (1). Current standard vital sign monitors include an ECG, periodic blood pressure recordings, and estimates of arterial oxygen-hemoglobin saturation. The need for a lifesaving intervention has been associated with systolic blood pressure of <90 mm Hg (24, 25); unfortunately, by the time these pressure reductions occur, it may already be too late to effectively resuscitate a bleeding patient. Cardiovascular collapse can occur quickly, and without advanced warning from standard vital signs. Because the autonomic nervous system is fundamentally responsible for maintaining stable hemodynamics, it is appropriate to focus on autonomic responses that change early. The results of the present investigation suggest that RRISD, pNN50, and RRI_{HF} may provide newly derived vital signs that could be obtained from ECG waveforms available on current medical monitors to assess the need for early intervention in advance of alterations in standard vital signs.

In addition to hemorrhage, it is likely that injury and pain associated with trauma may result in highly variable heart rate responses to trauma, unrelated to blood loss *per se*, hence reducing the sensitivity of heart rate as a predictor of patient outcome. This view is supported by a recent analysis of 10,825 patients with blunt or penetrating trauma, revealing that heart rate alone was neither sensitive nor specific in determining the need for emergent intervention (26). Although heart rate had an amalgamated correlation of 0.83 with MSNA during progressive LBNP in our subjects, RRI_{HF} correlated higher (0.98) with MSNA (Fig. 3) and changed earlier (LBNP at -30 mm Hg) (Fig. 2) than heart rate (LBNP at -45 mm Hg) (Table 1) during central hypovolemia. Our results therefore suggest that an index of sympathetic nerve activity may be more sensitive for assessment of blood loss in trauma patients than heart rate. This hypothesis is supported by the observation of significant differences in heart period variability between trauma patients who died compared with a cohort of patients who lived, despite their heart rates being similar (7, 8, 27). Our results from the present investigation are therefore consistent with those data obtained from the trauma literature suggesting that the use of heart

period variability may increase the sensitivity of decision-support tools for clinical assessment of bleeding patients.

Assessed in the time domain, increases or decreases of heart period variability are thought to reflect increases or decreases of the overall magnitude of autonomic influences on the heart, but contributions from either parasympathetic or sympathetic components cannot be determined (22). Nevertheless, based on thousands of individual bedside records from patients monitored continuously in the ICU, reductions of heart rate standard deviations assessed in bins of 1 to 4 secs are associated strongly with poor outcome (5, 6, 28). Whether poor patient outcomes assessed with periodic analysis of heart rate standard deviations are associated specifically with autonomic dysfunction has not been determined. In this regard, our experiment is unique in that it provided the opportunity to assess the relationship between heart period variability derived by time domain and direct measures of increasing sympathetic nerve activity in unanesthetized humans undergoing progressive reduction in central blood volume. Our results support the notion that periodic analysis of heart rate standard deviations can be used to assess autonomic dysfunction in bleeding patients and signal the need for early intervention.

Although autonomic cardiovascular control cannot be assessed directly in trauma patients (i.e., direct measures of MSNA), the results of our experiment suggest that autonomic function may be estimated in casualties with mild to severe hemorrhage through analysis of heart period variability in the frequency domain. In humans, cardiac interbeat intervals oscillate at two primary frequencies; high (respiratory) frequencies (0.15–0.4 Hz) and low frequencies (0.04–0.15 Hz). High-frequency oscillations are eliminated with large doses of atropine (29, 30), and low-frequency oscillations are reduced with propranolol (31, 32). Based largely on these autonomic blockade studies, RRI_{HF} oscillations have been attributed primarily to fluctuations of vagal nerve traffic, and RRI_{LF} oscillations have been attributed to a combination of factors, including fluctuations of both vagal and sympathetic traffic (9). Although RRI_{LF} oscillations are associated in some way with fluctuations of sympathetic neural activity (10, 33), it is clear that the association is imperfect (14, 15). Our results support this concept and challenge

the usefulness of RRI_{LF} as a tool to assess patient status during hemorrhage.

However, even those investigators who have championed the use of spectral analysis as an indicator of autonomic function concede that RRI_{LF} is most useful as an estimate of sympathetic nerve activity during procedures that progressively increase sympathetic traffic (9, 33, 34). Our results in the present study do not support this notion because LBNP failed to increase RRI_{LF} in even a single subject, despite progressive increases of MSNA (Figs. 2 and 3; 0% significant correlations between RRI_{LF} and MSNA; Table 2). It therefore seems likely that sympathetic compensation to central hypovolemia as occurs during hemorrhage cannot be adequately estimated or tracked directly with RRI_{LF} . After normalization of RRI_{LF} to express LF oscillations as a fraction of total power, normalized RRI_{LF} increases directly with MSNA during LBNP. However, because the normalization procedure involves dividing LF by LF+HF, and we observed that RRI_{HF} oscillations track changes of directly measured MSNA whereas RRI_{LF} changes do not, the fact that normalized RRI_{LF} tracks MSNA may simply reflect the influence of the relationship between RRI_{HF} and MSNA. Therefore, our experiment may be the first to demonstrate that RRI_{HF} represents the most specific frequency-derived heart period variability measure to reflect the autonomic response to hemorrhage.

Autonomic responses to simulated hemorrhage in our cohort of healthy subjects were appropriate as evidenced by a relative lack of change in mean arterial pressure. Compared with baseline, systolic pressure was reduced at the -45 mm Hg level, but the magnitude of change was small (about 7%), and systolic pressure never fell to <112 mm Hg on average, despite large reductions of central volume as estimated from pulse pressure (Table 1). In contrast, as early as the -30 mm Hg level, both pNN50 and RRI_{HF} were reduced by about 50% compared with baseline (Fig. 2). These data highlight the fact that autonomic function changes earlier than standard vital signs, such as arterial pressure during simulated hemorrhage, and suggest that tracking measures of autonomic function may provide unique insight into the clinical status of bleeding patients. Our data further indicate that the application of RRI_{HF} as a potential clinical tool for early assessment of blood loss compared with

measurements of standard vital signs can be applied universally to both male and female patients.

Without substantial anxiety, pain, or tissue injury, we are able to assess with confidence the isolated effect of progressive central hypovolemia. Our model of simulated hemorrhage necessarily induces early physiologic compensations to moderate or severe central hypovolemia, with severe hypotension observable at only the highest levels of negative pressures and just before cardiovascular collapse. In some severely injured patients, relative hypotension in the prehospital arena is sufficient to recommend immediate intervention (24), but by the time arterial pressures fall to dangerous levels, some patients may be unsalvageable. The inability to maintain adequate arterial pressures could be related importantly to autonomic decompensation. For example, Converse et al. (35) showed that hypotension induced by hemodialysis was associated with a relative parasympathetic activation and sympathetic withdrawal. Others have also reported parasympathetic predominance (estimated from an elevated HF/LF) in ICU patients who eventually died of their injuries (3, 4). In previous studies, we estimated autonomic function from heart period variability in prehospital trauma patients and found an elevated HF/LF in those patients who either eventually died of hemorrhage (7) or from all-cause trauma (8). In all these cases, an increase in parasympathetic predominance estimated from an elevation in RRI_{HF} was associated with increased mortality (i.e., a late indicator of outcome). The results from our present experiment are the first to suggest that progressive reductions in RRI_{HF} can provide an early indication of the sympathetic predominance associated with appropriate compensation during reduction in central blood volume before reaching a condition of irreversible patient outcome.

Our results also confirm that pulse pressure may provide insight into actual volume loss because it tracked the reduction in central blood volume. Convertino et al. (21) have shown that arterial pulse pressure provides a good indication of relative reductions in stroke volume during central hypovolemia. More important, our data suggest that indices of autonomic responses (e.g., RRI_{HF} , $RRISD$, and $pNN50$) may provide noninvasive decision support for actual circulating blood volume status. Because alterations

in RRI_{HF} , $RRISD$, and $pNN50$ are highly correlated with pulse pressure (all $R^2 \geq 0.88$), we hypothesized that heart period variability metrics should represent a sensitive and specific marker to track the effectiveness of fluid resuscitation and blood loss. Our LBNP model provided the opportunity to test this hypothesis because central blood volume was restored to baseline at the cessation of negative pressure. Indeed, restoration of the magnitude of heart period variability (Fig. 2) with concomitant restoration of pulse pressure (Table 1) at the cessation of LBNP suggests how heart period variability might also prove useful in monitoring adequate resuscitation during infusion of fluids. Thus, in addition to measurements of blood pressure, continuous monitoring of heart period variability may provide medical personnel insight into the status of central blood volume during bleeding and intervention.

Results of the current study should be interpreted with at least one technical caveat, and that is the practical utility of assessing heart period variability in real time. Due to the necessity of identifying R-wave fiducial points from the ECG with absolute certainty for frequency domain analyses, and given any confounding factors such as movement artifact, ectopic beats, or electrically noisy ECG recordings, R-R interval frequency domain analyses may not be possible for clinical applications until future monitors have the computing capability to filter, clean, and distinguish sinus node R-waves from non-sinus node sources. On the other hand, it seems from our analyses that simple time domain heart period variability metrics also track autonomic function well. Such analyses are more forgiving of ectopic or missing beats and may at this time be the most promising approach to assess autonomic function in the prehospital setting similar to what has been demonstrated in the ICU (5, 6, 28).

Study Limitations. We do not suggest that subjects in the present study resemble actual bleeding patients approaching hemorrhagic shock. Subjects in our experiment were not anxious or in pain, nor did they suffer tissue injury, and we therefore cannot speculate how the addition of these real-world issues might affect our results. Although we are unaware of any evidence to suggest that injury and pain would alter the fundamental physiologic relationship between heart period variability and sympathetic nerve activity observed in this study, we cannot dismiss

the possibility that trauma in addition to central blood loss could influence this relationship. A second potential limitation of the current study is the loss of nerve recordings at high levels of negative pressure. Due to inherent intricacies associated with obtaining direct measurements of MSNA in humans, coupled with movements of the microelectrode associated with LBNP, it is difficult to maintain adequate nerve recordings as chamber pressures increase. Despite this, to our knowledge, the MSNA data we present at -70 and -80 mm Hg are the only data available that provide insight into associations of heart period variability and sympathetic traffic during central hypovolemia of a magnitude similar to severe hemorrhage.

Clinical Implications. Monitoring and assessing circulatory volume status in bleeding patients from standard vital signs is problematic because vital signs change relatively late during injury progression. Monitoring of heart period variability may increase the capability to identify patients who are or are not compensating appropriately to hemorrhage. Potential uses include remote monitoring in mass-casualty situations, prehospital emergency transport (7, 8), ICU monitoring of septic patients (36) and patients undergoing renal dialysis (37) leading potentially to hypotension, and other conditions in which blood volume has the potential to decrease undetected. In the prehospital or military setting, tracking changes of autonomic function with heart period variability from a simple ECG could increase the potential to recognize a bleeding patient who seems stable but who may actually be progressing to cardiovascular collapse and death.

ACKNOWLEDGMENTS

We thank the subjects for their cheerful cooperation, Mr. Gary Muniz for excellent technical assistance, and Dr. John McManus for his assistance with physical examinations and medical monitoring.

REFERENCES

1. Carrico CJ, Holcomb JB, Chaudry IH: Post resuscitative and initial utility of life saving efforts: Scientific priorities and strategic planning for resuscitation research and life saving therapy following traumatic injury. Report of the PULSE trauma work group. *Acad Emerg Med* 2002; 9:621–626
2. Orlinsky M, Shoemaker W, Reis ED, et al: Current controversies in shock and resuscitation.

- tation. *Surg Clin North Am* 2001; 81: 1217–1262
3. Winchell RJ, Hoyt DB: Spectral analysis of heart rate variability in the ICU: A measure of autonomic function. *J Surg Res* 1996; 63: 11–16
4. Winchell RJ, Hoyt DB: Analysis of heart-rate variability: A noninvasive predictor of death and poor outcome in patients with severe head injury. *J Trauma* 1997; 43:927–933
5. Norris PR, Morris JA, Ozdas A, et al: Heart rate variability predicts trauma patient outcome as early as 12 h: Implications for military and civilian triage. *J Surg Res* 2005; 129:122–128
6. Grogan EL, Norris PR, Speroff T, et al: Volatility: A new vital sign identified using a novel bedside monitoring strategy. *J Trauma* 2005; 58:7–14
7. Cooke WH, Salinas J, Convertino VA, et al: Heart rate variability and its association with mortality in pre-hospital trauma patients. *J Trauma* 2006; 60:363–370
8. Cooke WH, Salinas J, McManus JG, et al: Heart period variability in trauma patients may predict mortality and allow remote triage. *Aviat Space Environ Med* 2006; 77: 1107–1112
9. Parati G, Mancia G, Di Rienzo M: Point: Cardiovascular variability is/is not an index of autonomic control of the circulation. *J Appl Physiol* 2006; 101:676–678
10. Pagani M, Montano N, Porta A, et al: Relationship between spectral components of cardiovascular variabilities and direct measures of muscle sympathetic nerve activity in humans. *Circulation* 1997; 95:1411–1448
11. Eckberg DL: Sympathovagal balance: A critical appraisal. *Circulation* 1997; 96: 3224–3232
12. Taylor JA, Studinger P: Counterpoint: Cardiovascular variability is not an index of autonomic control of the circulation. *J Appl Physiol* 2006; 101:678–681
13. Pagani M, Lombardi SG, Guzzetti S, et al: Power spectral analysis of heart rate and arterial pressure variabilities as a marker of sympatho-vagal interaction in man and conscious dog. *Circ Res* 1986; 59:178–193
14. Kingwell BA, Thompson JM, Kaye DM, et al: Heart rate spectral analysis, cardiac norepinephrine spillover, and muscle sympathetic nerve activity during human sympathetic nervous activation and failure. *Circulation* 1994; 90:234–240
15. Saul JP, Rea RF, Eckberg DL, et al: Heart rate and muscle sympathetic nerve variability during reflex changes of autonomic activity. *Am J Physiol* 1990; 258:H713–H721
16. Cooke WH, Ryan KL, Convertino VA: Lower body negative pressure as a model to study progression to acute hemorrhagic shock in humans. *J Appl Physiol* 2004; 96:1249–1261
17. Cooke WH, Convertino VA: Heart rate variability and spontaneous baroreflex sequences: Implications for autonomic monitoring during hemorrhage. *J Trauma* 2005; 58:798–805
18. Butler GC, Yamamoto Y, Xing HC, et al: Heart rate variability and fractal dimension during orthostatic challenges. *J Appl Physiol* 1993; 75:2602–2612
19. Victor RG, Leimbach N: Effects of lower body negative pressure on sympathetic discharge to leg muscles in humans. *J Appl Physiol* 1987; 63:2558–2562
20. Hagbarth KE, Vallbo AB: Pulse and respiratory grouping of sympathetic impulses in human muscle nerves. *Acta Physiol Scand* 1968; 74:96–108
21. Convertino VA, Cooke WH, Holcomb JB: Arterial pulse pressure and its association with reduced stroke volume during progressive central hypovolemia. *J Trauma* 2006; 61: 629–634
22. Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology: Heart rate variability: Standards of measurement, physiological interpretation, and clinical use. *Circulation* 1996; 93:1043–1065
23. Ludwig DA: Use and misuse of p-values in designed and observational studies: Guide for researchers and reviewers. *Aviat Space Environ Med* 2005; 76:675–680
24. Franklin GA, Boaz PW, Spain DA, et al: Pre-hospital hypotension as a valid indicator of trauma team activation. *J Trauma* 2000; 48: 1034–1039
25. Holcomb JB, Niles SE, Miller CC, et al: Pre-hospital physiologic data and life saving interventions in trauma patients. *Mil Med* 2005; 170:7–13
26. Brasel KJ, Guse C, Gentilello LM, et al: Heart rate: Is it truly a vital sign? *J Trauma* 2007; 62:812–817
27. Batchinsky AI, Cancio LC, Salinas J, et al: Prehospital loss of R-to-R interval complexity is associated with mortality in trauma patients. *J Trauma* 2007; 63:512–518
28. Norris PR, Ozdas A, Cao H, et al: Cardiac uncoupling and heart rate variability stratify ICU patients by mortality: A study of 2088 trauma patients. *Ann Surg* 2006; 243: 804–814
29. Pomeranz B, Macaulay RJB, Caudill MA, et al: Assessment of autonomic function in humans by heart rate spectral analysis. *Am J Physiol* 1985; 248:H151–H153
30. Fouad FM, Tarazi RC, Rerrario CM, et al: Assessment of parasympathetic control of heart rate by a noninvasive method. *Am J Physiol* 1984; 246:H838–H842
31. Saul JP, Berger RD, Albrecht P, et al: Transfer function analysis of the circulation: Unique insights into cardiovascular regulation. *Am J Physiol* 1991; 261:H1231–H1245
32. Saul JP, Berger RD, Chen MH, et al: Transfer function analysis of autonomic regulation: II. Respiratory sinus arrhythmia. *Am J Physiol* 1989; 256:H153–H161
33. Malliani A, Pagani M, Lombardi F, et al: Cardiovascular neural regulation explored in the frequency domain. *Circulation* 1991; 84: 482–492
34. Malliani A: The pattern of sympathovagal balance explored in the frequency domain. *News Physiol Sci* 1999; 14:111–117
35. Converse RL, Jacobsen TN, Jost CM, et al: Paradoxical withdrawal of reflex vasoconstriction as a cause of hemodialysis-induced hypotension. *J Clin Invest* 1992; 90: 1657–1665
36. Ellenby MS, McNames J, Lai S, et al: Uncoupling and recoupling of autonomic regulation of the heart beat in pediatric septic shock. *Shock* 2001; 16:274–277
37. Cashion AK, Cowan PA, Milstead EJ, et al: Heart rate variability, mortality, and exercise in patients with end-stage renal disease. *Prog Transplant* 2000; 10:10–16